Quebrachitol–A New Component of Maple Sap and Sirup

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Quebrachitol, a cyclic polyol (1-*O*-methyl-L-inositol), was identified for the first time from maple sap and found more abundant than all monosaccharides combined. Fructose and glucose combined accounted for less than 0.1% of the dry weight of the sap. Quebrachitol was also isolated from maple sirup. The concentration of quebrachitol decreased slightly during the late winter and spring months until the trees broke dormancy in April, when there was an abrupt drop. Maple sap was gathered during the sap season from January to mid-April in a manner that prevented possible enzymic or microbial hydrolysis of saccharides and was analyzed for carbohydrates by a gas chromatographic method.

Maple sirup is produced by evaporating the sap of the sugar maple, the species of hard maple common in the Northeastern states and the Great Lakes region. The sap used for this purpose is generally gathered from the trees during the late winter and early spring.

The sap collected from the maple tree is colorless and flavorless. The color and flavor of the sirup are developed by heating in the presence of air during the evaporation process (Willits and Porter, 1950). Both the color and flavor vary considerably in maple sirups produced during the different periods of the sap-gathering season.

Previous work from this laboratory has shown that the alkaline degradation of simple sugars present in maple sap may be responsible for development of color and flavor during the evaporation process (Naghski and Willits, 1957; Stinson and Willits, 1965). Sucrose is the predominant component of maple sap and sirup, but other sugars are present in trace amounts. These include mono-, di-, and trisaccharides as well as high oligosaccharides (Bois and Nadeau, 1938; Haq and Adams, 1961; Porter *et al.*, 1954; Watanabe and Aso, 1962).

The Eastern Regional Laboratory is concerned with factors causing variation in flavor and color in maple sirup. During an investigation of the changes that occur in the carbohydrate composition of maple sirup in a process for making highly flavored maple sirup (Willits *et al.*, 1966), gas chromatography indicated the presence of a polyhydroxy compound previously undetected as a constituent of maple sap or sirup. However, Plouvier (1948) reported this compound as a constituent of maple tree bark. This polyhydroxy compound is present in amounts greater than either fructose or glucose, and in quantity it ranks next after sucrose.

This paper describes the isolation and identification of this compound as quebrachitol, and methods for measuring the concentrations of quebrachitol, fructose, and glucose in sap, and follows their concentration throughout the sap collection season.

Materials and Methods

Maple Sap and Sirup. Maple sap used in this investigation was obtained during the months of January, March, and April of 1964 from a single tree growing on the laboratory grounds at Wyndmoor, Pa. The tree was tapped by an aseptic technique described by Naghski and Willits in 1955. The sap used for the isolation of the unknown was collected in a 5-gallon translucent plastic bag which transmitted sufficient ultraviolet radiation to prevent bacterial growth (Naghski and Willits, 1953). This sap was collected each day that sap flowed. The sap was immediately dried by first partially concentrating it by vacuum evaporation in a rotating flask heated at 25° C. in a water bath; absolute ethanol was added to form an azeotrope with the remaining water and the slurry evaporated as before. The solid residue was stored at −29° C.

The sap for gas chromatographic analysis was frozen at the tree site immediately as it left the tree; the sap passed through a short length of plastic tubing attached to the outlet of the same spile to a test tube immersed in dry ice and was frozen within a few seconds. These frozen samples were stored at −29° C. Comparison of samples analyzed immediately after collection and samples analyzed after several months of storage indicated that no breakdown of saccharides had occurred during storage. The maple sirup used for isolation of the unknown material was obtained from a commercial producer in central New York.

Chemical Reagents. Fructose, glucose, acetic anhydride, sodium acetate, and pyridine, all reagent grade, were obtained from Fisher Scientific Co., and used without further purification. Pyridine, once opened, was stored over KOH pellets until used. Sucrose and sorbitol, reagent grade, obtained from the same source, were purified by recrystallizing from water-ethanol and ethanol, respectively, before use.

Hexamethyldisilazane and trimethylchlorosilane were obtained from the Peninsular Chemical Co., Gainesville, Fla., and used without further purification.

Quebrachitol, 1-*O*-methyl-L-inositol, was obtained from Calbiochem, Los Angeles, Calif., and used without further purification.

Gas Chromatographic Reagents. Carbowax 20M, Apiezon L, SE-52, and Gas Chrom Z, 60- to 70-mesh, were obtained from Applied Science Laboratories, State College, Pa.

Gas Liquid Chromatography. APPARATUS. Gas chromatograph, F & M Model 810-12, Avondale, Pa., equipped with dual columns, thermal conductivity and hydrogen flame ionization detectors, and isothermal and linear temperature programming. Recorder, −0.2

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to 1 mv., Model ElectroniK 16, Honeywell, Philadelphia, Pa.

COLUMNS. Coiled stainless steel columns, 3.66 feet × 0.25-inch o.d., containing 4.2% SE-52, 4.5% Carbowax 20M, and 3.0% Apiezon L on Gas Chrom Z support, 60 to 70 mesh, were prepared in this laboratory using the solution coating technique to prepare the stationary phase (Horning *et al.*, 1963). All analytical work was performed on the SE-52 columns. The other columns were used for identification by cochromatography.

CONDITIONS. Helium as carrier gas, 60 ml. per minute, 48 pounds' pressure at inlet, atmospheric pressure at outlet; hydrogen, approximately 65 ml. per minute; compressed air, approximately 500 ml. per minute; detection and injection chambers, 300° C. Temperature of the column chamber was maintained isothermally at 140° C. for 14 minutes, followed by a linear temperature increase of 4° C. per minute, to 230° C. This temperature was maintained for 15 minutes. The columns were cooled to 140° C. prior to injection of another sample.

Derivatives for Gas Chromatography. TRIMETHYLSILYL ETHERS, prepared using the trimethylsilation procedure described by Sweeley *et al.* (1963). Sap samples containing 10 mg. of total solids were transferred to 1-dram plastic-stoppered vials and evaporated to apparent dryness under an air stream, and then to complete dryness in a desiccator under water aspirator vacuum. The dry residues were dissolved in 1.0 ml. of pyridine, and 0.2 and 0.1 ml. of hexamethyldisilazane and trimethylchlorosilane, respectively, were added. The vials were stoppered, shaken vigorously until the contents had completely dissolved, and allowed to stand a minimum of 10 minutes at room temperature, after which the reaction was considered complete. The derivatives were stable, as samples analyzed after several days had elapsed showed no alteration. Aliquots of the reaction mixtures were injected directly into the gas chromatograph for analysis using the flame ionization detector.

ACETATES, prepared by the sodium acetate acetylation procedure of Contardi (1945).

Preparative Gas Chromatography. Larger quantities of the acetate and trimethylsilyl ether derivatives of the compound isolated from sap and of pure quebrachitol were purified for infrared, nuclear magnetic resonance, and mass spectrographic analysis by isothermal gas chromatography at 140° C., using the SE-52 columns and the thermal conductivity detector. The compounds were trapped in capillary melting point tubes inserted into the exit port of the gas chromatograph.

Qualitative Chemical Tests. OXIMATION AND SODIUM BOROHYDRIDE REACTION. Tests of the purified compound were made using the procedure described by Sweeley (1963). After drying the reaction mixtures, the trimethylsilyl ethers were prepared and examined by gas liquid chromatography for evidence of reaction.

SCHERER QUALITATIVE TEST FOR INOSITOLS. Quebrachitol isolated from sap, together with known quebrachitol, L-inositol, and sucrose as comparison standards were examined by Feigl's modification of the Scherer test for inositols (Feigl, 1956).

Instrumental Analysis. INFRARED SPECTRA. The infrared spectra of solid films of quebrachitol from sap and commercial quebrachitol were obtained on thallium bromide–iodide windows (Ard, 1964) using a Beckman IR-7 instrument. The infrared spectra of the trimethylsilyl ether derivatives of quebrachitol from maple sap and from commercial quebrachitol were obtained by placing the samples between NaCl windows.

NUCLEAR MAGNETIC RESONANCE. The trimethylsilyl ether derivatives were analyzed using a Varian DP-60 instrument.

MASS SPECTRA. The trimethylsilyl ether derivative of quebrachitol from sap was analyzed on the Model 21-103 C mass spectrograph manufactured by the Consolidated Electrodynamics Corp.

Results and Discussion

Isolation of Quebrachitol from Maple Sap and Sirup. Maple sap was selected for initial isolation of the unknown monosaccharide after preliminary experiments showed that the compound was more abundant in sap than in maple sirup. The sap also contained fewer extraneous materials that would complicate purification.

Ninety-five grams of dried solids from 1 gallon of sap was dissolved in 35 ml. of water. Absolute ethanol was added to bring the concentration to 85% ethanol (v./v.). The solution was permitted to stand for 30 minutes to allow most of the sucrose to be crystallized and removed by filtration.

Gas chromatography showed that this filterable material was nearly pure sucrose, while the filtrate contained the unknown component and reducing sugars.

The filtrate was evaporated to dryness under vacuum. The solids were then dissolved in a minimum amount of water and were precipitated with alcohol. This process was repeated four times. The final precipitation yielded 0.99 gram of a solid mixture estimated by gas chromatography, on the basis of the relative peak heights, to consist principally of quebrachitol with a small amount of sucrose and trace amounts of other unidentified material.

Several recrystallizations of the impure mixture from ethanol–water, 3 to 2, resulted in 140 mg. of pure quebrachitol. The same procedure was used to isolate quebrachitol from maple sirup.

Identification as Quebrachitol. The identity of the compound was easily established by instrumental analysis. The infrared spectra of the purified compound indicated strong absorption in the regions typical for hydroxyl groups, and moderate absorption in the region typical of ethers, but gave no indication of the presence of unsaturation. The absence of either free or potential carbonyl groups was also indicated by the failure of the compound to form oximes or sugar alcohols as indicated by the unaltered gas chromatographic behavior of the trimethylsilated reaction mixtures. The infrared spectra of the trimethylsilyl derivative of the unknown compound showed absorption in the region associated with ether linkages. The trimethylsilyl ether derivative decomposed during mass spectral analysis so that the molecular weight could not

be obtained. However, numerous fragments containing C—O—CH_3 residues were obtained, indicating that the original compound contained at least one methyl ether group. No triplet or quartet peaks were apparent in the nuclear magnetic resonance spectra of the trimethylsilyl derivative, indicating the absence of methylene or methyl groups adjacent to the —CH groups.

These data would be consistent with a saturated cyclic compound having either a hydroxyl or methoxy substituent at each position of the ring. Acyclic compounds containing only hydroxyl or methoxy substituents were eliminated, as these compounds would possess either methylene or methyl groups on the terminal carbon atoms. The possibility of a naturally occurring acyclic diacetal was eliminated, as the compound was stable in acid.

The Scherer test, specific for inositols, confirmed the presence of an inositol compound. The compound isolated from sap gave a pink color matching the color produced by known quebrachitol, while inositol produced a red color.

The identity of the unknown compound with known quebrachitol was confirmed by the mixed melting point (unaltered at 189–90° C.) and identical infrared spectra. The C and H calculated for $C_7H_{14}O_6$ (quebrachitol) is C, 43.30%; H, 7.27%. Found by C and H combustion: C, 43.38%; H, 7.19%. Additional confirmation was given by identical infrared spectra of the trimethylsilyl ethers, the mixed melting point of the acetates of both known quebrachitol and that compound isolated from sap (unaltered at 90–91° C.), and cochromatography of the acetates and trimethylsilyl ethers of both known quebrachitol and quebrachitol from maple sap and sirup on SE-30, SE-52, Carbowax 20M, and Apiezon L columns.

Quantitative Analytical Procedure. A quantitative method for measuring the quantities of carbohydrates present in maple sap was necessary to detect the changes in carbohydrate composition during the sap season.

The method described in this paper converts the sugars and quebrachitol into their trimethylsilyl ethers for analysis by gas chromatography. Following the recommendations of Dal Nogare (1962) peak heights rather than peak areas were used to measure the concentrations of the components of sap owing to the small quantities of glucose and fructose present. Linear temperature programming was used to increase the sharpness and intensity of the peaks. An internal standard, D-sorbitol, was used to minimize variations in response owing to the performance of the instrument and sample size.

Calibration of Instrument. Calibration charts (Figure 1) were prepared showing the response of the instrument to fructose, glucose, and quebrachitol. Two series of solutions, each containing 10.0 mg. of solids in 1.00 ml. of water, were used. The solutes of the first series consisted of 1, 2, 3, 4, and 5%, respectively, of quebrachitol with the remainder sucrose. The solutes of the second series contained 0.05, 0.10, and 0.20% each of fructose and glucose, with the remainder sucrose. One-milliliter aliquots were transferred by pipet to vials, evaporated to dryness, and dissolved in

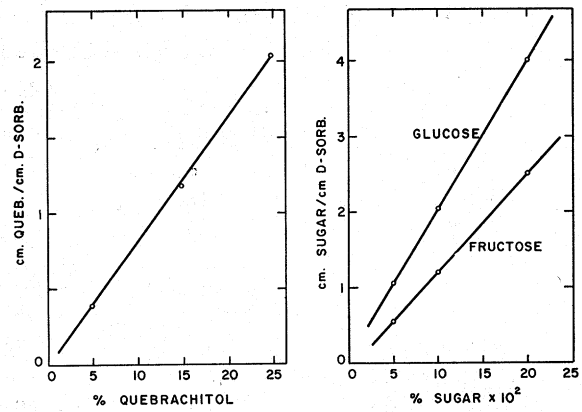

Figure 1. Calibration of instrument

1.00 ml. of pyridine containing D-sorbitol, and the trimethylsilyl ether derivatives were prepared. The concentration of D-sorbitol for the quebrachitol standard was 0.100 gram of D-sorbitol per 500 ml. of pyridine. The standard for the glucose and fructose determination was 5.00 ml. of the quebrachitol standard solution diluted with anhydrous pyridine to 200.0 ml.

Aliquots containing up to several microliters of the reaction mixture were injected directly into the gas chromatograph equipped with the SE-52 columns at 140° C. This temperature was maintained for 10 minutes, followed by linear temperature programming at 4° per minute to 240° C. The solvent and excess reagents emerged at 140° C., while the carbohydrates emerged during programming. The order of emergence was fructose at 160°, quebrachitol at 169°, α-glucose at 172°, D-sorbitol at 176°, and β-glucose at 182° C. Heating the column at 240° C. for a short time permitted removal of sucrose and other polysaccharides.

The calibration charts in Figure 1 were prepared by calculating the ratio between the height of the peak produced by the compound and the height of the peak produced by the internal standard, and plotting this ratio against the per cent of the compound in the sample. For glucose, the combined heights of the peaks produced by the alpha and beta anomers were used.

Analysis of Sap. The stored frozen samples of sap were melted and 5-ml. portions transferred to weighing bottles in which they were evaporated to dryness in two stages as described above and weighed. The residues were dissolved in water, transferred to 10.0-ml. volumetric flasks, and diluted to the mark. Aliquots corresponding to 10.0 mg. of solid matter were transferred to 1-dram vials and the derivatives prepared and chromatographed in the same manner used in the calibration series. The concentration of the different components in the sap was determined by comparing the ratio between the heights of the respective peaks and the sorbitol peak with the corresponding value on the calibration chart. This permitted direct determination of the concentration of the different components in the sap.

Accuracy of Method. The accuracy of the procedure was checked by the recovery method. Known amounts of quebrachitol and glucose were added to aliquots of sap samples that had been analyzed previously. The

Table I. Recovery of D-Glucose and Quebrachitol Added to Maple Sap

	Total Glucose Found, μg.	Glucose Present from Sample, μg.	Glucose Found	Glucose Added	Estimated Recovery, %
Glucose 1	21	12	9	10	90
Glucose 2	18	9	9	10	90
Glucose 3	25	15	10	10	100
	Total Quebrachitol Found, μg.	Quebrachitol Present from Sample, μg.	Quebrachitol, μg. Found	Quebrachitol, μg. Added	Recovery, %
Quebrachitol 1	400	208	192	200	96
Quebrachitol 2	474	276	198	200	99
Quebrachitol 3	404	212	192	200	96

Table II. Concentration Changes during Sap Season

Date	Constituents of Sap: Fructose, mg./100 ml. (% of solids)	Glucose, mg./100 ml. (% of solids)	Quebrachitol, mg./100 ml. (% of solids)	Total solids, g./100 ml.
Early (2/16/65)	0.96 (0.04)	2.88 (0.12)	137 (5.7)	2.4
Middle (3/16/65)	1.56 (0.06)	4.94 (0.19)	112 (4.3)	2.6
Late (4/9/65)	1.50 (0.05)	3.75 (0.13)	95 (3.8)	2.5

analytical procedures were repeated, and the amounts of quebrachitol and glucose recovered were compared to the amounts that had been added. The recovery of quebrachitol was between 96 and 99%. The recovery of glucose was between 90 and 100%. The data are given in Table I.

The results in Table II show that fructose and glucose are present only in trace amounts and that no clear pattern exists for their concentration throughout the sap season. Their presence at the concentrations found may be an artifact arising from incidental hydrolysis of sucrose. Quebrachitol, however, is a true component of maple sap and although it exists in small amounts, it represents the second major constituent of sap after sucrose. Table II shows a definite decrease in quebrachitol concentration in sap as the season progresses.

Conclusions

A general correlation exists between the reducing sugar content of maple sap and the formation of color and flavor during the evaporation process. Both properties tend to become more pronounced as the season progresses and the reducing sugar concentration increases. The low concentrations of fructose and glucose and the absence of any discernible trend in their concentration make it improbable that fructose or glucose is the main factor in determining the formation of color and flavor during the evaporation of sterile sap to maple sirup. Fructose and glucose may be involved in the formation of color and flavor during the evaporation of nonsterile sap to sirup.

The chemical stability of quebrachitol and its decreasing concentration as the season progresses make it rather unlikely that it decomposes during the ordinary sirup manufacturing process to any appreciable extent or participates in the reactions that result in development of color and flavor in sirup. These properties are ordinarily greatest in late season sirup when the quebrachitol concentration is lowest.

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